Mold exposure during infancy as a predictor of potential asthma development

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Background: Exposure to mold has been associated with exacerbation of asthma symptoms in children.

Objective: To report how the presence of visible mold and exposure to (1–3)-β-D-glucan in infancy affects the risk of asthma at the age of 3 years as defined by an Asthma Predictive Index (API).

Methods: Visible mold was evaluated by means of home inspection. (1–3)-β-D-glucan levels were measured in settled dust. Children were considered to be at high risk for asthma at later ages if they reported recurrent wheezing at the age of 3 years and met at least 1 of 3 major or 2 of 3 minor API criteria.

Results: Children aged 3 years with high visible mold in the home during infancy were 7 times more likely to have a positive API than were those with no visible mold (adjusted odds ratio [aOR], 7.1; 95% confidence interval [CI], 2.2–12.6). In contrast, whereas those with high (1–3)-β-D-glucan levels (≥22 µg/g) were at increased risk of a positive API (aOR, 3.4; 95% CI, 0.5–23.5), those with high (1–3)-β-D-glucan levels (≥133 µg/g) were at decreased risk (aOR, 0.6; 95% CI, 0.2–1.6). Of the other covariates, mother’s smoking was the strongest significant risk factor for the future development of asthma based on a positive API (aOR, 4.4; 95% CI, 1.7–11.6).

Conclusions: The presence of high visible mold and mother’s smoking during infancy were the strongest risk factors for a positive API at the age of 3 years, suggesting an increased risk of asthma. High (1–3)-β-D-glucan exposure seems to have an opposite effect on API than does visible mold.

INTRODUCTION

Several studies1,2 have shown that home dampness and visible mold are associated with the severity of respiratory symptoms, such as wheezing, coughing, and asthma, in children. The association between mold exposure and the development of asthma is less studied. Parental report of a moldy odor at home in the past year has been associated with the development of physician-diagnosed asthma in children aged 1 to 7 years.3 Similarly, parental reports of visible mold or moisture at home during the child’s first 12 months were significantly associated with wheeze and asthma in children aged 9 to 11 years.4 More recently, homes of 1- to 7-year-old children with new clinically determined asthma (with ≥2 attacks of wheezing) and their controls were inspected by trained civil engineers. Moisture damage, visible mold, and higher levels of viable mesophilic actinomycetes in the main living quarters were associated with increased risk of physician-diagnosed asthma.5,6 However, exposure to fungal components, such as extracellular polysaccharides and (1–3)-β-D-glucan, early in life has been shown to decrease the risk of parental report of physician-diagnosed asthma and sensitization to inhalant allergens in children.7–9 Endotoxin, a bacterial component that may be elevated in moldy buildings, has inconsistently been associated with either increased or decreased frequency of wheezing or physician-diagnosed asthma in early childhood.7,8,10

Studies11,12 of the natural history of asthma have demonstrated that in most cases of persistent asthma, the initial asthma-like symptoms occur during the first several years of life. We examined the association between environmental exposures and the development of atopy and atopic respiratory disorders (including asthma and rhinitis) in a birth cohort of children of atopic parents.13 At the age of 1 year, exposure to mold and environmental tobacco smoke (ETS) was associated with increased recurrent wheezing.14,15 In contrast, exposure to a high level of fungal (1–3)-β-D-glucan and high endotoxin exposure in the presence of multiple dogs were associated with decreased recurrent wheeze and allergen sensitization in infants.15,16 Because health outcomes associated with exposures in infant populations may be transient, this study examines how exposure to mold and (1–3)-β-D-glucan in infancy predicts the risk of future asthma in this birth cohort.

METHODS

Recruitment

Infants were identified from birth certificate records (October 1, 2001, through July 31, 2003) and were recruited into the Cincinnati Childhood Allergy and Air Pollution Study as described previously. This study was approved by the institutional review boards of the University of Cincinnati and Cincinnati Children’s Hospital Medical Center. Informed consent was obtained from a parent of each participating child.

Exposure Evaluation

When infants reached an average age of 8 months, a detailed questionnaire was administered to the parents regarding home characteristics. Dust samples were vacuumed from the floor of the child’s primary activity room. Each room, including the basement, was visually inspected for signs of mold or water damage. The location of the damage, changes in the color and integrity of the surface material, and the size of the damaged surface were recorded. Tape samples were collected from suspect surfaces to verify mold contamination by microscopic examination as described by Heinsohn and Reponen. The extent of home mold and water damage was categorized as none, low (moldy odor or moisture damage or visible mold area <0.2 m²), and high (moisture damage and visible mold area ≥0.2 m²). The criteria for home classification were developed by Meklin et al and were based on International Society of Indoor Air Quality and Climate guidelines for mold cleanup.

Concentrations of (1–3)-β-D-glucan and endotoxin in dust samples were determined via the end point chromogenic Limulus amebocyte lysate assay using Glucatell modification for (1–3)-β-D-glucan and Pyrochrome for endotoxin (Associates of Cape Cod, East Falmouth, Massachusetts), as described by Issisifova et al and Campo et al.

Medical Evaluation

Infants were evaluated during an annual physician’s office visit beginning at the age of 1 year. The results reported herein include health outcomes at the age of 3 years. Infants were tested for allergen sensitization using skin prick tests (SPTs) to a panel of food (milk and egg) and 15 common indoor and outdoor aeroallergens (cat, dog, German cockroach, house dust mite, 7 pollens, and 4 mold antigens [Alternaria tenuis, Aspergillus fumigatus, Penicillium notatum, and Cladosporium herbarum]). Those with a positive SPT reaction to any allergen (aeroallergen or food allergen) were defined as SPT(±), those SPT(±) to only 1 or more aeroallergens were defined as SPTAero(±), and those SPT(±) to at least 1 mold were defined as SPTmold(±). Children who experienced 2 or more wheezing episodes in the previous 12 months and were SPT(+) were classified as having recurrent wheeze with allergen sensitization. This health outcome is hereafter referred to as “wheezing in children with atopy.” Children who had only 1 or no wheezing episodes in the past 12 months and had negative SPT results served as the comparison group.

Children aged 3 years were classified as having a high risk of future asthma based on an Asthma Predictive Index (API) proposed by Castro-Rodriguez et al and modified by Gilbert et al. The API was initially developed for children at the age of 3 years and had a positive predictive value for asthma at the ages of 6 to 13 years of 59.1%, a negative predictive value of 73.2%, a specificity of 84.7%, and a sensitivity of 41.6%. For this analysis, children were considered to have a positive API, ie, at high risk of asthma at later ages, if they were reported to have recurrent wheezing at the age of 3 years and met at least 1 of 3 major criteria (parental asthma history, allergic sensitization to ≥1 aeroallergens, and eczema) or 2 of 3 minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg). Recurrent wheezing was defined as 2 or more wheezing episodes in the previous 12 months. Eczema was defined as either physician-diagnosed eczema on physical examination or parental report of frequent skin scratching for more than 6 months accompanied by red spots, raised bumps, or rough, dry, scaly skin.

Data Analysis

The descriptive statistics for mold, (1–3)-β-D-glucan, and endotoxin levels and their association with health outcomes at the age of 1 year have been reported previously. The present article presents the association between exposure at the age of 1 year and health outcomes at the age of 3 years. The API was the major focus, but wheezing in children with atopy and SPT were also analyzed as separate health outcomes. The analysis of API and SPT outcomes included 483 children who had exposure information and medical evaluation. The wheezing analysis included a subset of 285 children, with 44 having wheezing with SPT(+) and 241 having no report of wheezing and SPT(+). The remainder of the group (n = 198) had either SPT(+) or wheezing and were not included when analyzing the wheeze outcome.

Wheeze, API, and SPT results were analyzed using multiple logistic regression analysis. Adjusted odds ratios (aORs) for each health outcome regarding visible mold in the home were obtained for the 3 categories of mold (none, low, and high, with none as the baseline to which each higher level was compared). Histograms and quantile-quantile plots showed that (1–3)-β-D-glucan and endotoxin levels were approximately log-normally distributed and were, therefore, log-transformed and continuously modeled in analyses. The aORs and 95% confidence intervals (CIs) for (1–3)-β-D-glucan and endotoxin levels were compared. Histograms and quantile-quantile plots showed that (1–3)-β-D-glucan were obtained to estimate the odds of each outcome for an infant in each quartile. The reference value was the lowest end point at each quartile.

Predictor variables and exposures that can affect asthma development at a later age were included in the univariate analyses, and those significant at the 20% level in the univariate analyses were then included in the multivariate logistic regression analyses. Variables that maintained sig-
Significance levels approximately equal to 5% in at least 1 wheeze or API model or that changed the regression coefficient (or SE) of another variable by at least 15% when dropped from the model were kept in the final model. Visible mold, (1–3)-β-D-glucan, and endotoxin remained a priori in all models. Predictor variables that were evaluated but not included in the final model were breastfeeding duration (<1, 1–24, ≥25 weeks), dust mite and cockroach allergens, and number of dogs and cats in the home. The predictor variables evaluated at the age of 1 year that were categorically modeled were race (black vs nonblack), number of siblings in the household (1 vs 0, >1 vs 0), mother’s smoking (≥20 vs 0 cigarettes daily), lower respiratory tract symptoms (≥1 vs 0 of whooping cough, croup, viral infections, bronchitis/bronchiolitis, influenza, and pneumonia), and upper respiratory tract symptoms (≥1 vs 0 of cold, ear infection, sinus infection, strep throat, tonsilitis, and colored drainage).

Owing to the relatively small number of children in the category of high mold levels in the home (primary exposure variable) — 11 and 16 for atopic wheezing and API, respectively—a sensitivity analysis was undertaken to help ensure that no single person had undue influence on the results. The influence on model variables was evaluated by the removal of a different randomly chosen child in the high visible mold exposure category for the analysis of both outcomes. The analyses were obtained using S-Plus software (Insightful Corp, Seattle, Washington).

RESULTS

Exposure and Participant Characteristics

Of the 483 children in this study, 16% were black, 43% were male, and 32% had no siblings in the home (Table 1). Almost half of the children reporting high visible mold at home (7 of 16) had a positive API, and one-third (4 of 11) had atopic wheezing. Overall, one-fifth of those with any visible mold had a positive API and atopic wheezing (Table 1). One-third of children whose mothers smoked (18 of 58) and one-quarter of those who had lower respiratory tract symptoms (45 of 178) had a positive API (Table 1). Of 78 children with a positive API and 44 atopic children with wheezing, more than 75% had 1 or more upper respiratory tract symptoms, and more than two-thirds of this high-risk group was male. Of the total cohort, 203 children (42.0%) had aeroallergen sensitization and 19 (3.9%) had mold sensitization. No associations were found between SPT results and the presence of visible mold at home.

Health Outcomes at the Age of 3 Years

The aORs for wheezing in children with atopy and API are given in Table 2. Children with high visible mold at home were 6 times more likely to have wheezing with atopy and 7 times more likely to have a positive API than were those without visible mold. These associations were significant even with a small number of children in the high mold category (n = 16). Sensitivity analysis showed no change in any variable estimate for either outcome when randomly chosen individuals were removed from the high mold exposure category. For lower mold exposure, findings remained elevated but nonsignificant for both outcomes.

Findings were reversed for (1–3)-β-D-glucan levels. As (1–3)-β-D-glucan exposure levels increased, the aOR for wheezing in children with atopy decreased from the first to the fourth quartile (Table 2). There was a similar trend of decreasing risk of a positive API (Table 2). Similar findings were seen regarding (1–3)-β-D-glucan levels and risk of SPT (+). Exposure to low levels of (1–3)-β-D-glucan (0.35–22.0 μg/g) at the age of 1 year was associated with 3 times higher risk of SPT (+) (aOR, 3.39; 95% CI, 0.99–11.53) at the age of 3 years. When those SPT (+) only to food were excluded, this finding was largely attributed to being SPT (+) to an Aeroallergen (aOR, 3.40; 95% CI, 0.99–11.73). Again, the risk was progressively lower with the increase in (1–3)-β-D-glucan exposure (133.1–960.0 μg/g; SPT (+): aOR, 0.54; 95% CI, 0.28–1.02; SPTaero (+): aOR, 0.52; 95% CI, 0.27–1.00) (data not shown).

Endotoxin exposure was associated with a slightly increased risk of wheezing in children with atopy and a positive API, but this was not statistically significant when tested on a continuous scale or in quartiles. Of the other covariates, mother’s smoking was the strongest significant risk factor for wheezing in children with atopy (aOR, 13.7) and future development of asthma based on a positive API (aOR, 4.4).

Black race and having lower or upper respiratory tract symptoms were also significant risk factors for wheezing in children with atopy and a positive API (Table 2).

DISCUSSION

We previously presented data on environmental exposures, including visible mold and (1–3)-β-D-glucan, and related sensitization and wheezing in this cohort at the age of 1 year. Herein we report the effect of these exposures in the same cohort at the age of 3 years. Furthermore, the main focus, and the uniqueness, of this study is that it is, to our knowledge, the first to explore the effect of visible mold and (1–3)-β-D-glucan exposure on the clinical API. We found that exposure to high visible mold before the age of 1 year is a significant risk factor for a positive API at the age of 3 years. This indicates a significantly increased risk of asthma as children with a positive API have been shown to be 2.6 to 5.5 times more likely to have active asthma between the ages of 6 and 13 years than children with a negative API. A similar positive association was found between visible mold and wheezing in children with atopy.

Although there is sufficient evidence for an association between visible mold and exacerbation of asthma symptoms, few studies have investigated whether exposure to dampness or visible mold also causes asthma in young children. Jaakkola et al13 and Rydjord et al14 relied on self-reported exposure data, whereas Pekkanen et al15 used a standardized home inspection to record visible mold in homes of newly diagnosed asthma cases. The present study, using standardized evaluation of visible mold, prospectively demonstrates...
that exposure to high visible mold during the first year of life is associated with higher risk of future asthma. This study also shows that an increase in exposure to high (1–3)-β-D-glucan concentrations (>133.1 μg/g) decreased the risk of future asthma based on the API. Similar trends were seen for wheezing and SPT outcomes. These findings, although not statistically significant, support a previous finding of a statistically significant inverse association between

<table>
<thead>
<tr>
<th>Characteristic at age 1 y</th>
<th>Wheezing in children with atopy* at the age of 3 y</th>
<th>Positive API at the age of 3 y</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Children, No. (n = 285)</td>
<td>Symptom prevalence, No. (%) (n = 44; 15.4%)</td>
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<tr>
<td>Race</td>
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<tr>
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<td>All others</td>
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<tr>
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</tr>
<tr>
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<td>36 (17.7)</td>
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<tr>
<td>Female</td>
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<td>No. of siblings</td>
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<tr>
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<td>1</td>
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<td>≥2</td>
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<tr>
<td>Lower respiratory tract symptomsb</td>
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<td>Yes</td>
<td>107</td>
<td>27 (25.2)</td>
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<tr>
<td>Upper respiratory tract symptomsb</td>
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<td>Mother’s smoking</td>
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<td>Yes</td>
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<td>11 (25.6)</td>
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<tr>
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<td>33 (13.6)</td>
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<td>Visible mold</td>
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<td>Low (&lt;0.2 m²)</td>
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<tr>
<td>High (&gt;0.2 m²)</td>
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<td>4 (36.4)</td>
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<td>Parental asthma†</td>
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<tr>
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<td>14 (13.4)</td>
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<tr>
<td>(1–3)-β-D-glucan quartile, μg/g</td>
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<tr>
<td>I: 0.35–22.0</td>
<td>70</td>
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<td>II: 22.1–60.0</td>
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<td>III: 60.1–133.0</td>
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<td>Endotoxin quartile, EU/mg‡</td>
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<tr>
<td>I: 6.0–38.8</td>
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<td>SPT(+) at age 3 y</td>
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<tr>
<td>SPTaero(+)</td>
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</tr>
<tr>
<td>SPTmold(+)</td>
<td>18</td>
<td>18 (100)</td>
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Abbreviations: API, Asthma Predictive Index; EU, endotoxin unit; SPT, skin prick test.

*Wheezing in children with atopy was defined as 2 or more wheezing episodes in the past 12 months and a positive SPT reaction. Children who had 1 or no wheezing episodes in the past 12 months and were SPT negative were used as the comparison group.

†Lower respiratory tract symptoms include whooping cough, croup, viral infections, bronchitis/bronchiolitis, influenza, and pneumonia.

‡Upper respiratory tract symptoms include cold, ear infection, sinus infection, strep throat (positive culture), tonsillitis, and colored drainage.

Parental asthma was not included as a predictor variable for API because it is used to define API.

Five samples were missing for wheezing in children with atopy at the age of 3 years.
(1–3)-β-D-glucan exposure and recurrent wheezing at the age of 1 year. An association between (1–3)-β-D-glucan exposure and an increased T_{H1} immune response has been suggested and is supported by findings of an inverse relationship with (1–3)-β-D-glucan and sensitization to inhalant allergens in children aged 2 to 4 years and by parental report of physician-diagnosed asthma and persistent wheeze in children aged 1 to 4 years. In contrast to high (1–3)-β-D-glucan levels, low levels were associated with higher risk of allergen sensitization or wheeze. Similarly, epidemiologic studies have revealed a biphasic pattern in which sensitization risk increases with exposure levels until a plateau is reached, above which risk decreases with further increase in exposure.

The opposite effects on API between high visible mold and high fungal glucan levels may be attributed to many factors. It is likely that (1–3)-β-D-glucan only partially represents the effects of the total indoor mold exposure and that other fungal components, such as peptidoglycans, extracellular polysaccharides, toxins, and allergens, add to the differences between studies. For example, inverse relationships have been reported between extracellular polysaccharides in house dust and wheezing outcomes in farm children and parental report of physician-diagnosed asthma. High bacterial N-acetylmuramic acid concentrations in mattress dust were associated with a lower frequency of wheezing and possibly asthma in rural schoolchildren. However, most fungal spores are known to contain, or with germination to produce, allergens. Furthermore, IgE-mediated sensitization to fungi such as Alternaria, Aspergillus, Cladosporium, and Penicillium species is a strong risk factor for asthma. Exposures to these fungi during the first year of life have been associated with physician-diagnosed lower respiratory tract illnesses, subsequent wheezing, and persistent cough in infants of asthmatic parents or having asthmatic siblings. Therefore, high visible mold exposure and asthma development in children cannot be taken as a simple cause-and-effect relationship.

In addition, glucan sources include not only visible mold in the home but also hidden mold, outdoor fungi, some soil bacteria, and plant material, eg, pollen and cellulose. Indoor fungal species also vary widely in their (1–3)-β-D-glucan content. Species known to be highly allergenic, such as Aspergillus and Alternaria, have been shown to have low (1–3)-β-D-glucan content. Thus, (1–3)-β-D-glucan may not be a good indicator of mold contamination in a building but may be an independent measure of biologically active exposure.

Endotoxin did not have significant associations with any of the studied health outcomes. Although the inverse association between endotoxin and atopic wheeze was confirmed in several European studies, after adjustment for (1–3)-β-D-glucans and extracellular polysaccharides, the effect of endotoxin was no longer significant. A recent study showed that household endotoxin exposure was not associated with
asthma or wheezing in children. In other urban cohort studies, higher endotoxin levels in house dust during the first year of life were associated with a higher prevalence of wheeze at the ages of 1 through 3 years. Unlike many of these previous studies, in this cohort, we did not observe a correlation among visible mold, (1–3)-β-D-glucan, and endotoxin levels. Therefore, we propose that (1–3)-β-D-glucan and mold may have opposite risks for a positive API.

The adverse effects of mother’s smoking, living with siblings, and having lower respiratory tract symptoms observed at the age of 1 year in this cohort were still present at the age of 3 years. In this study, mother’s smoking was the second strongest predictor of future asthma based on a positive API. It is well known that prenatal maternal smoking and child ETS exposure are associated with decreased lung function and growth. Although there is consistent evidence that exposure to ETS exacerbates asthma in children, research on a causal relationship between ETS exposure and asthma induction is insufficient, and the mechanism has not yet been established.

A potential limitation of this study is the early age at outcome measurement. Many children who wheeze before the age of 3 years have transient episodes and do not develop clinical asthma later in life. Furthermore, 25% to 40% of children who have a positive API will never have active asthma during their school years. However, this chosen age had an up to 5-fold increased risk of asthma by the age of 13 years. Therefore, continued follow-up of this birth cohort is necessary to determine whether the findings at the age of 3 years will translate into a decreased risk of asthma later in life. Furthermore, future studies are needed to better define the complex role of simultaneous exposure to different fungal components in the development of allergic respiratory diseases.

In conclusion, visible mold and mother’s smoking were the strongest risk factors for the future potential development of asthma based on a positive API. Thus, preventive measures for asthma development, which could be effective in high-risk children, are home remediation measures to remove visible mold and parental smoking cessation.

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